Interaction of Digoxin and Montmorillonite: Mechanism of Adsorption and Degradation

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Received June 23, 1978, from the *Departments of *Industrial and Physical Pharmacy, ‡Bionucleonics, and §Agronomy, Purdue University, West Lafayette, IN 47907.* Accepted for publication August 31, 1978.

Abstract □ IR, X-ray diffraction, and adsorption studies showed that digoxin is adsorbed onto montmorillonite by a reversible adsorption mechanism at pH 2 and 6. Degradation studies indicated abnormally high acid hydrolysis rates for digoxin interacted with montmorillonite. Accelerated digoxin degradation is attributed to the ability of the clay surface to concentrate both digoxin and protons. The effective pH at the clay surface appeared to be 1.5 pH units lower than the bulk suspension pH. Bisdigoxigenin was the major adsorbed degradation product. A similar catalytic effect also may occur with other neutral drugs that degrade by acid hydrolysis and should be considered in the formulation of clay-containing drug products or their coadministration with other drugs.

Keyphrases □ Digoxin—interaction with montmorillonite, mechanism of adsorption and degradation, IR, X-ray diffraction, and adsorption studies □ Montmorillonite—interaction with digoxin, mechanism of adsorption and degradation, IR, X-ray diffraction, and adsorption studies □ Interactions—digoxin and montmorillonite, mechanism of adsorption and degradation, IR, X-ray diffraction, and adsorption studies

The adsorption mechanism of the cationic drugs clindamycin and tetracycline by montmorillonite was elucidated recently using IR and X-ray analysis (1). Both drugs were adsorbed by cation exchange under pH conditions favoring the cationic form of the drug. Tetracycline also was found to complex with certain interlayer cations at higher pH conditions where the zwitterionic or anionic forms were present.

The application of IR spectroscopy and X-ray diffraction to the study of drug–clay interactions was extended to neutral compounds using digoxin as the model drug. Interaction was possible since many neutral molecules are known to interact with clays by physical adsorption (2) and hydrogen bonding (3). In addition, a number of saccharides similar to digitoxose have been shown to interact with montmorillonite (4).

Montmorillonite was used as a model clay because it possesses the highest surface area and exchange capacity of the clays commonly used in pharmacy. Since these properties are related directly to clay adsorption mechanisms, the results obtained with montmorillonite should be useful in predicting the adsorption behavior of clay minerals having lower adsorptive capacities such as kaolinite and attapulgite.

EXPERIMENTAL

Materials—All chemicals were either official or reagent grade. An X-ray diffractogram of bentonite USP indicated that it was composed of montmorillonite and a small quantity of quartz. To study the clay component responsible for the adsorptive properties observed in bentonite USP, the <2-μm clay fraction was separated by sedimentation and collected (1).

The cation-exchange capacity of the purified montmorillonite was 102 mEq/100 g as determined by the ammonium saturation method (5). Of this total, metal-ion analysis by atomic absorption spectrometry[1] showed that 7.1% of the cation-exchange capacity was satisfied by calcium ions, 2.7% by magnesium ions, and the balance by sodium ions.

Table I—Changes (in Angstroms) of Interlayer Spacing of Montmorillonite Interacted with Digoxin

	pH 2.0		pH 6.0	
Washings	Digoxin–Clay	Clay Control	Digoxin–Clay	Clay Control
1	4.6, 0.2–3.0	0.9	4.6	1.4
5	1.3	1.3	5.4	1.2
10	1.3, 2.5	1.2	4.9	1.3

Adsorption Mechanism—One milliliter of digoxin stock solution [60 mg/ml in chloroform–ethanol (1:1)] was added to clay aliquots and adjusted with hydrochloric acid or sodium hydroxide to pH 2.0 or 6.0 to yield 100 ml of a 1% montmorillonite suspension. After mixing and correcting for any pH change, the clay–drug system was equilibrated for 1 hr at 37°. Aliquots were then centrifuged at 6000 rpm, the supernate was decanted, and the solids were resuspended in water adjusted to the initial mixture pH.

To each washing was added enough chloroform–ethanol to achieve the original 1% (v/v) ratio of solvent to suspension. After 15 min of equilibration, this procedure was repeated to give samples washed 1, 5, and 10 times. For these samples, clay–drug solids were resuspended in water to yield a 2% suspension. IR and X-ray analyses of these clay–drug samples were then performed (1).

Effect of pH on Adsorption—Three adsorbate solutions containing 0.2 mg of digoxin were prepared from a digoxin stock solution (0.02 mg/ml in 50% ethanol) and labeled with 2.0 μCi of ^{3}H-digoxin[2] (specific activity of 12.8 mCi/mmole) in 100 μl of benzene–ethanol (1:9). These solutions were then added to clay suspensions containing 1 g of montmorillonite. The drug–clay mixtures were diluted to 1% clay suspensions and adjusted to pH 2, 4, and 6. Interaction occurred at 37° over a 5-hr period.

Aliquots were centrifuged for 30 min at 6000 rpm. Supernatant liquids were decanted into a second test tube to which a few crystals of sodium chloride were added to facilitate separation of the clay. The equilibrium concentration of the supernate was determined by quantitating the radioactivity using liquid scintillation counting[3] techniques. The counting efficiency was established using an external standard, and all samples were counted to a preset counting error of ±2.0%.

Effect of Montmorillonite on Digoxin Degradation—Montmorillonite and digoxin samples were prepared as already described except that pH adjustments were made to 2.0, 3.0, 4.0, and 5.0, and 40 μCi (2.0 ml) of the ^{3}H-digoxin stock solution was incorporated into each unlabeled digoxin solution. After immediate centrifugation for 30 min at 6000 rpm, each sample was decanted, the solids were washed twice with chloroform–methanol (1:1), and the two wash supernates were combined.

This double extraction, together with the first supernate, showed 99 ± 9% recovery of the tritium label. Subsequent evaporation under a dry nitrogen stream to the aliquot volume yielded a concentrated radioactive solution representing adsorbed digoxin and any adsorbed degradation products.

Labeled digoxin control samples were prepared without clay at pH 1.0, 1.5, 2.0, 2.5, and 3.0. Degradation was halted in each aliquot by adding 1 *N* NaOH to bring the pH above 3.0.

Twenty microliters of the desorbed (extracted) solutions and the control aliquots were spotted on precoated silica gel TLC plates[4] and developed in ethyl acetate–chloroform–acetic acid (90:5:5) (6). A chlo-

[1] Model 290B, Perkin-Elmer Corp., Norwalk, Conn.

[2] ^{3}H-Digoxin, Amersham Corp., Arlington Heights, Ill.

[3] Isocap/300 liquid scintillation system (Searle Analytical, Des Plaines, Ill.) with Riafluor (New England Nuclear, Boston, Mass.) as the scintillant.

[4] E. Merck AG, Darmstadt, West Germany.

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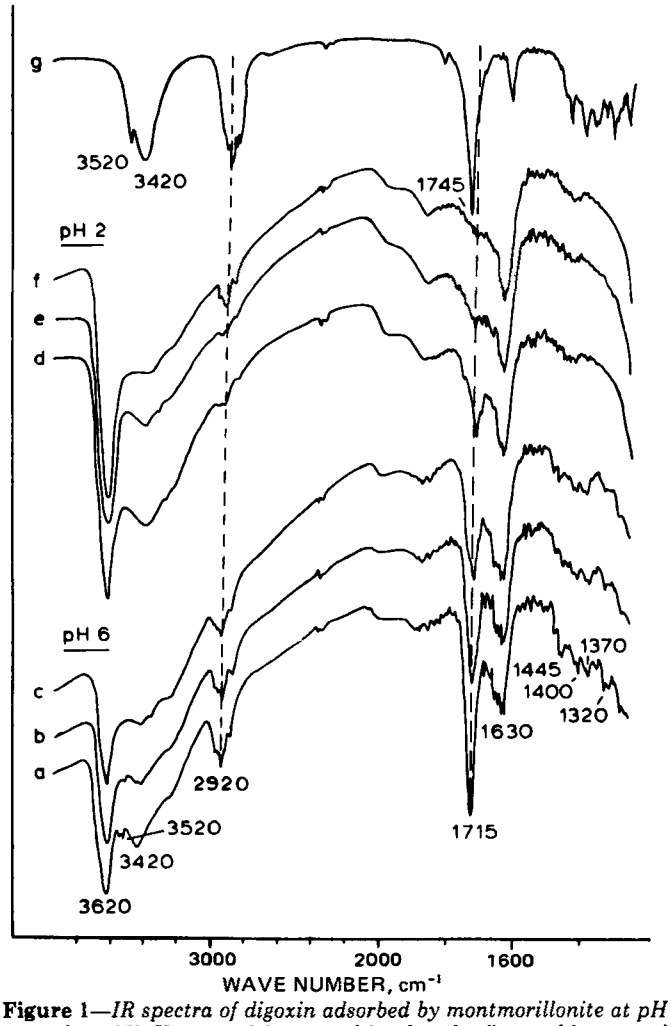

Figure 1—*IR spectra of digoxin adsorbed by montmorillonite at pH 2.0 and 6.0, 37°. Key: a and d, one washing; b and e, five washings; c and f, 10 washings; and g, digoxin in potassium bromide.*

roform–isopropanol–acetone (80:5:15) system (6) confirmed that total separation of digoxin and its degradation products was attained with the first system.

The concentration of each component was determined by measuring the radioactivity level in 5-mm sections of the TLC plates. Each zone was scraped into a scintillation vial, mixed with scintillant, and counted. The components were identified by matching experimental R_f values with those of standard compounds.

RESULTS

X-Ray—The X-ray diffraction d_{001} spacing for montmorillonite in the absence of drug under the dehydration conditions used in this study was 9.6 Å, indicating that no interlayer molecules (*i.e.*, water or drug) were present in the control sample. Adsorption of drug molecules between the layers of montmorillonite increased the d_{001} spacing; the difference between the d_{001} spacing of the expanded montmorillonite–drug system and the d_{001} spacing of the collapsed montmorillonite–control system is designated as the Δd_{001} value. The Δd_{001} values for digoxin–montmorillonite samples at pH 2.0 and 6.0 are listed in Table I.

The minimum dimension of the drug was calculated to be 5.6 Å from a Corey–Pauling–Kolton (CPK) molecular model. Keying of the digoxin molecule in the oxygen surface of the montmorillonite layers may result in an apparent contact distance that is 1 Å less than the true molecular dimension (7). This foreshortening agrees well with the experimental values reported in Table I.

The Δd_{001} values due to digoxin interaction at pH 2.0 (Table I) differ from those at pH 6.0. Although a Δd_{001} of 4.6 Å was observed after one washing for both pH 6.0 and 2.0, the pH 2.0 peak contained a shoulder indicating additional Δd_{001} values in the 0.2–3.0-Å range. After five washings, only a Δd_{001} value of 1.3 Å was detected. This Δd_{001} value was due most likely to nonaqueous solvent adsorption and was verified by an identical spacing in the control sample (Table I) where chloroform–methanol (1:1) was added without digoxin. This 1.3-Å value is considerably less than the thickness of a single layer of solvent molecules and was probably due to irregular surface coverage. A Δd_{001} of 1.3 Å was again observed after 10 washings and was accompanied by a large shoulder indicating a concomitant Δd_{001} value of 2.5 Å. This result can be attributed to more complete and uniform solvent adsorption.

The decrease in the Δd_{001} value from 4.6 to 1.3 Å as a result of washing indicated that digoxin was no longer present between the clay layers and that simple washing facilitated desorption. Such behavior implies physical adsorption or hydrogen bonding as the means of digoxin–montmorillonite interaction. X-ray diffraction patterns of samples at pH 2.0 were more broad and of lower intensity than those observed at pH 6.0. This finding suggests irregularly expanded clay sheets due to incomplete adsorption. The diffraction peaks of samples at pH 6.0 were very sharp and indicated a high degree of intercalation.

IR—IR spectra for digoxin adsorbed by montmorillonite at pH 2.0 and 6.0 are shown in Fig. 1. The absorption band at 3620 cm^{-1} was assigned to the OH-stretching vibration of the clay; water displayed an OH-asymmetric stretching band at 3420 cm^{-1} and an OH-bending vibration at 1630 cm^{-1}. Digoxin absorption bands were evident at 3520, 3420, 2920, and 1715 cm^{-1} and in the 1445–1320-cm^{-1} region. With repeated washings, all drug band intensities decreased (Fig. 1, curves b, c, e, and f), indicating that desorption occurred readily. This reversible behavior is characteristic of weak bonding forces (8).

The IR spectra of digoxin–montmorillonite support weak adsorption mechanisms. Generally, adsorption due to hydrogen bonding can be detected by shifts in the absorption bands of participating functional groups. Numerous hydroxyl groups, especially those in the digitoxoside portion of the digoxin molecule, might be expected to take part in hydrogen bonding with the clay surface. Although these OH-stretching vibrations at 3520 and 3420 cm^{-1} were readily apparent in the pH 6.0 clay–drug sample, no shifts in frequency were noted (Fig. 1, curve a).

Physical adsorption would be expected to result in isolated molecules of digoxin on the clay surface, thus reducing intermolecular hydrogen bonding between digoxin molecules (9, 10). Decreased hydrogen bonding causes stretching vibrations to shift to a higher frequency. Since this was not observed for the drug hydroxide bands (Fig. 1, curve a), it is suggested that hydrogen bonding between digoxin and the clay surface occurs but that the energy levels involved are comparable to intermolecular bonding in digoxin (Fig. 1, curve g). The digoxin carbonyl-stretching vibration shifted from 1745 to 1715 cm^{-1} as a result of interaction with montmorillonite. This shift to lower frequency further indicates hydrogen bonding as the principal adsorption mechanism, although van der Waals' forces also may contribute.

At pH 2.0, the low concentration of adsorbed digoxin and the interference from the OH-stretching frequency of water obscured any possible shifts in the OH-stretching vibrations of the drug as a result of interaction with montmorillonite. However, a shift in the carbonyl vibration from 1745 to 1715 cm^{-1} was apparent (Fig. 1, curve d).

Although the adsorption mechanism seemed to be the same for samples at pH 2.0 and 6.0, their relative intensities were quite different; the pH 2.0 bands were substantially smaller than the corresponding ones at pH 6.0. Since digoxin degrades by acid hydrolysis with the apparent first-order rate constant increasing below pH 3.0 (11–14), the smaller amount of adsorbate observed by IR and X-ray analysis at pH 2.0 was thought to be due to digoxin degradation. Therefore, the effect of pH on adsorption and degradation was investigated.

Effect of pH on Adsorption—Adsorption studies of tritiated digoxin were run at pH 6.0, 4.0, and 2.0 (Table II). The nearly complete adsorption seen at pH 6.0 was expected based on the high ratio of adsorbent to adsorbate. At this pH, no acid hydrolysis occurred and the percent digoxin adsorbed remained essentially constant at 90%.

At pH 4.0, the initial adsorption of 87% decreased to 51% after 5 hr.

Table II—Effect of pH on Adsorption of Digoxin and Digoxin Degradation Products

Hours	Tritium Label Adsorbed, %		
	pH 6	pH 4	pH 2
0	91	87	81
0.5	91	83	24
1	90	80	24
2	91	70	25
5	92	51	22

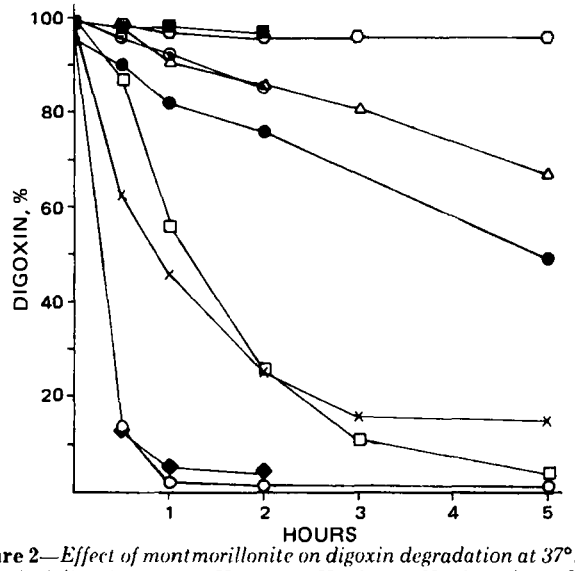

Figure 2—*Effect of montmorillonite on digoxin degradation at 37°. Key (adsorbed digoxin at): ○, pH 2.0; □, pH 3.0; △, pH 4.0; and ○, pH 5.0. Key (digoxin control at): ♦, pH 1.0; ×, pH 1.5; ●, pH 2.0; ⊙, pH 2.5; and ■, pH 3.0.*

This result was unexpected since minimal degradation has been reported at pH 4.0 (11–14) and no desorption was expected under the experimental conditions.

Interaction at pH 2.0 showed the immediate adsorption of 81% of the tritium label. This amount decreased rapidly to 24% within 30 min. The observed decrease in adsorption may have been due to digoxin degradation at this pH, although the apparent rate of change in adsorption was greater than would be predicted from the published digoxin hydrolysis rate at pH 2.0 (11–14). Because of this unexpected catalysis at pH 2.0 and 4.0, digoxin degradation in the presence of montmorillonite was examined in more detail.

Effect of Montmorillonite on Digoxin Degradation—Tritiated digoxin and montmorillonite were interacted at pH 2.0, 3.0, 4.0, and 5.0 and sampled over 5 hr. The adsorbed species (digoxin and its degradation products) were extracted from the montmorillonite, separated, and quantified by TLC and liquid scintillation counting. The percent digoxin in the adsorbate is presented in Fig. 2; the percent bisdigoxigenin, the initial digoxin degradation product, is shown in Fig. 3.

The bisdigoxigenin formation rate at pH 2.0, 3.0, and 4.0 indicates accelerated digoxin degradation. Montmorillonite did not alter the kinetics of digoxin degradation, as evidenced by the first-order relationship obtained at each pH. However, the degradation rate was significantly

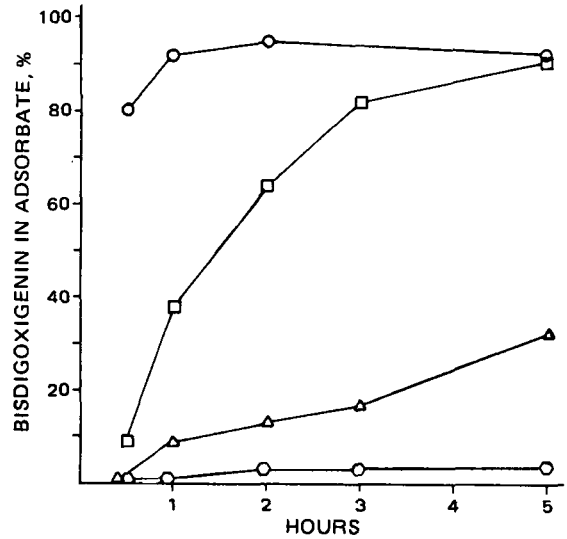

Figure 3—*Effect of montmorillonite on bisdigoxigenin formation at 37°. Key (adsorbed digoxin at): ○, pH 2.0; □, pH 3.0; △, pH 4.0; and ○, pH 5.0.*

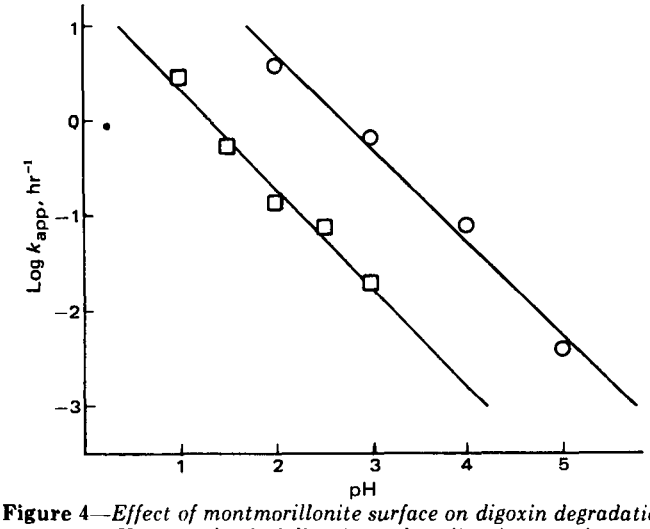

Figure 4—*Effect of montmorillonite surface on digoxin degradation rate at 37°. Key: ○, adsorbed digoxin; and □, digoxin control.*

increased (Fig. 4). The rate constants for the controls (Fig. 2) agreed well with previous studies (12–14).

DISCUSSION

After 1 hr of interaction with montmorillonite at pH 2.0, the adsorbed digoxin had degraded 98% (Fig. 2). However, 24% of the labeled compound remained adsorbed (Table II). Clearly, this latter adsorbate contains more than digoxin, probably a digoxin degradation product similar to digoxin in structure and thus accounting for the apparent digoxin bands in the IR spectra (Fig. 1, curve d). This suggestion is possible since the degradation pathway for digoxin acid hydrolysis involves cleavage of digitoxose molecules, yielding bisdigoxigenin, monodigoxigenin, and the digoxin aglycone, digoxigenin.

To identify the adsorbed degradation product responsible for the IR spectra at pH 2.0, relative quantities of all degradation products were obtained from the TLC and liquid scintillation counting analysis of digoxin adsorption. Figure 3 shows that bisdigoxigenin was the principal adsorbed component at pH 2.0. The smaller adsorption of bisdigoxigenin compared to that of digoxin at pH 6.0 was probably due to loss of a digitoxose unit, similar to other sugars known to be adsorbed by clays (4). Monodigoxigenin and digoxigenin also are generated under these conditions but have an even lower affinity for the clay due to digitoxose cleavage.

The lower degree of bisdigoxigenin adsorption by montmorillonite is illustrated by the X-ray diffraction data at pH 2.0 (Table I). After one washing, a Δd_{001} value of 4.6 Å was observed; however, the pattern was broader and of lower intensity than that observed at pH 6.0. Since the minimum molecular dimension of bisdigoxigenin is the same as digoxin, the Δd_{001} value would be unaffected by digoxin degradation. Collapse of the clay layers after five washings was a direct result of the weaker bonding between bisdigoxigenin and montmorillonite. With desorption facilitated by bisdigoxigenin formation, the nonaqueous solvent was then free to penetrate the clay layers to form a more complete and uniform solvent layer, ultimately resulting in a Δd_{001} value of 2.5 Å.

In the presence of montmorillonite, the hydrolysis rate suggests that the microenvironment of the clay is more acidic than the bulk solution. For example, Fig. 4 shows that the apparent rate constant at pH 4.0 for adsorbed digoxin was the same as the rate constant for a digoxin solution at pH 2.5. Although clays have been reported to catalyze the acid hydrolysis of esters such as ethyl acetate, the inversion of sucrose, and the protonation and hydrolysis of *s*-triazine herbicides (15, 16), the catalytic effect of clays on drugs is generally unrecognized.

This catalytic effect of montmorillonite on the acid-catalyzed digoxin degradation is due to the ability of the clay surface to serve as a site where digoxin molecules and protons are concentrated by adsorption and cation exchange, respectively. Therefore, the probability of reaction is enhanced, and an increased hydrolysis rate is observed. This effect was noted most dramatically at pH 2.0 (Table I) where 82% of the digoxin in the control remained after 1 hr at 37° while only 2% intact digoxin was found when interacted with montmorillonite.

A recent report suggested that some variation in the bioavailability

of orally administered digoxin may be due to variation in gastric pH, which would modify the composition of digoxin species available for absorption (12). The catalytic effect of montmorillonite will accentuate this problem. Therefore, the concomitant administration of drug products containing digoxin and montmorillonite should be avoided.

A similar catalytic effect may also occur with other neutral drugs that degrade by acid hydrolysis and should be considered in the formulation of clay-containing drug products or their coadministration with other drugs.

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ACKNOWLEDGMENTS

Supported in part by The Upjohn Co.

This report is Journal Paper 7215, Purdue University Agricultural Experiment Station, West Lafayette, IN 47907.

Digoxin was kindly supplied by the Burroughs-Wellcome Co.

NOTES

Crystal Structure of 1:1 Complex of Barbital with 1-Methylimidazole

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Received May 8, 1978, from the *Crystallography Department, University of Pittsburgh, Pittsburgh, PA 15260.* Accepted for publication August 9, 1978.

Abstract □ The prediction of a strong hydrogen-bonding interaction between barbital and 1-methylimidazole was confirmed. Two crystal complexes were obtained, 1:1 and 2:1, and the X-ray structure was determined for the 1:1 complex, which is monoclinic, space group $P2_1/c$, with a = 12.236(3) Å, b = 11.332(4) Å, c = 12.495(4) Å, and β = 120.67(1)°. The structure contains disk-shaped hydrogen-bonded tetramers with two molecules of each kind. There is a short NH···N hydrogen bond (2.82 Å) in which barbiturate provides the NH donor.

Keyphrases □ Barbital—complex with 1-methylimidazole, crystal structure determined □ 1-Methylimidazole—complex with barbital, crystal structure determined □ Complexes—barbital with 1-methylimidazole, crystal structure determined □ Crystal structure—determined for complex of barbital with 1-methylimidazole

Barbiturate drugs behave as strong hydrogen-bonding donors through their two NH groups but are weak acceptors through their three carbonyl oxygen atoms. These conclusions were derived from the hydrogen-bonding arrangements and interatomic distances observed in the crystal structures of a series of complexes of barbital with other small molecules representative of biological systems (1, 2). Thus, barbiturate drug receptor sites are likely to involve strongly electronegative hydrogen-bonding acceptor groups, such as the nitrogen atoms of adenine (3, 4), the imidazole ring in histidine or histamine (2), or the phosphoryl oxygen atoms of phospholipids (5).

These studies indicated that a strong hydrogen-bonding interaction would occur between barbital (5,5-diethylbarbituric acid) and 1-methylimidazole (Fig. 1), which might lead to crystal complexation. Indeed, 1-methylimidazole was a powerful solvent for barbital. The high viscosity of this solution made direct crystallization impractical. However, two crystal complexes were obtained from an ethanol solution; in one complex (1:1), crystal structure determination confirmed a short hydrogen bond NH···N (2.82 Å) in which barbital is the donor and 1-methylimidazole is the acceptor.

EXPERIMENTAL

Crystals of the 1:1 and 2:1 complexes were obtained from the same ethanolic solution, which was saturated at 60° with respect to both barbital and 1-methylimidazole, and slowly cooled in a sealed vial. Space groups and approximate cell data for these complexes were determined from X-ray precession photographs[1].

Crystals of the 1:1 complex, which dissociate at 96°, are monoclinic, space group $P2_1/c$, with a = 12.236(3) Å, b = 11.332(4) Å, c = 12.495(4) Å, and β = 120.67(1)°. The crystal density (1.190 g/cm^3) determined by

[1] No further work is planned on the 2:1 complex of barbital and 1-methylimidazole. These crystals are monoclinic, space group $P2_1/c$, with a = 16.7 Å, b = 12.1 Å, c = 12.4 Å, and β = 102°. There are eight barbital and four 1-methylimidazole molecules per unit cell.

0022-3549/79/0300-0361$01.00/0